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Pogostemon jaitapurensis (Lamiaceae), a new species from India based on morphological and molecular evidence

ARUN NIVRUTTI CHANDORE[1,2,7*], ASIF SHABODIN TAMBOLI[3,8*], DEVIDAS BHAUSAHEB BORUDE[1,2,9], AVINASH RAMCHANDRA GHOLAVE[4,10], SANJAY PRABHU GOVINDWAR[3,5,11] & SHRIRANG RAMACHANDRA YADAV [6,12]

[1] *Department of Botany, Abasaheb Marathe Arts and New Commerce, Science College, Rajapur-416 702, Maharashtra, India.*
[2] *University of Mumbai, Mumbai, India.*
[3] *Department of Biochemistry, Shivaji University, Kohapur-416 004, Maharashtra, India.*
[4] *Department of Botany, K.V.N. Naik Art's, Commerce and Science College, Nashik-422 002, Maharashtra, India.*
[5] *Department of Earth Resources and Environmental Engineering, Hanyang University, Seoul-04763, South Korea.*
[6] *Department of Botany, Shivaji University, Kolhapur-416 004, Maharashtra, India.*
[7] *arunchandore@gmail.com; https://orcid.org/0000-0003-0176-1840*
[8] *asift456@gmail.com; https://orcid.org/0000-0003-2146-670X*
[9] *devidasborude30@gmail.com; https://orcid.org/0000-0003-1114-4432*
[10] *agholave@gmail.com; https://orcid.org/0000-0002-8192-0102*
[11] *spgovindwar@hanyang.ac.kr; https://orcid.org/0000-0002-2098-6538*
[12] *sryadavdu@gmail.com; https://orcid.org/0000-0002-3528-9604*
**Authors contributed equally to this paper*
**Authors for correspondence: arunchandore@gmail.com, asift456@gmail.com*

Abstract

Based on morphological and molecular analyses, *Pogostemon jaitapurensis* is described and illustrated as a new species from Konkan region of Maharashtra, India. Present study added the molecular data of Indian endemic *Pogostemon* species of sect. *Verticillatus* and provide their phylogenetic placement. The bayesian and parsimony phylogenetic analyses conducted resolve *Pogostemon* into two clades, from them the new species belongs to Clade B and shows close relationship with species of sect. *Verticillatus*. A possible relationship between the new species and *P. deccanensis* and *P. erectus*, endemic to India, is also revealed by the phylogenetic analyses. Phylogenetically, the new species has been also placed in genus *Pogostemon* subg. *Dysophyllus* sect. *Verticillatus*. Sectional classification of these species is congruent with their phylogenetic positions.

Keywords: cpDNA; ITS, *Dysophylla*, Konkan, Lateritic Plateaus, Lamioideae, Pogostemoneae

Introduction

The genus *Pogostemon* Desfontaines (1815: 154) belongs to family Lamiaceae with 86 species distributed mainly in tropical and subtropical regions of Asia, Australia and Africa (Cook 1996, Bhatti & Ingrouille 1997, Pradeep 1998, Mabberley 2008, Yao *et al.* 2015). The highest species diversity is found in the Indian subcontinent (Bhatti & Ingrouille 1997) and about 41 species are reported from India, of which 19 are endemic (Sampathkumar *et al.* 2004, Sunojkumar *et al.* 2014). Yao *et al.* (2016) revised the infrageneric classification of the genus proposed by Bhatti & Ingroulle (1997) by presenting a comprehensive phylogenetic analysis in which they employed five chloroplast and one nuclear marker; dividing *Pogostemon* as follows:

1 subg. *Pogostemon* Desfontaines (1815: 154)
2 subg. *Allopogostemon* Bhatti & Ingrouille (1997: 97)
2.1 sect. *Racemosus* (Bentham 1848: 154) Bhatti & Ingrouille (1997: 97)
2.1.1 subsect. *Racemosus* Bhatti & Ingrouille (1997: 97)
2.1.2 subsect. *Glabriusculus* (Briquet 1897: 328) Bhatti & Ingrouille (1997: 102)
2.2 sect. *Zygocalyx* Bhatti & Ingrouille (1997: 104)

3 subg. *Dysophyllus* (Blume 1826: 826) Bhatti & Ingrouille (1997: 107)

3.1 sect. *Dysophyllus* Bhatti & Ingrouille (1997: 107)

3.2 sect. *Verticillatus* (Bentham 1848: 157) Bhatti & Ingrouille (1997: 111)

In general, *Pogostemon* can be distinguished from other Lamiaceae by the presence of exserted stamens bearing moniliform hairs (Hooker 1885, Kuntze 1891, Press 1982, Bhatti & Ingrouille 1997). Traditionally, most taxonomists have treated the genus as two separate groups (even as separate genera) based primarily upon habitat: the terrestrial group *Pogostemon sensu stricto* (s.s.) and the aquatic/marshland group *Dysophylla* Blume (1826: 826) (Bentham 1833, Hooker 1885, Doan 1936, Airy Shaw 1967, Li & Hedge 1994, Yao *et al.* 2016). Taxa within the terrestrial group generally possess opposite leaves that are broad and petiolate, and have comparatively large flowers (calyx usually longer than 3 mm, corolla usually longer than 4 mm). Taxa within the aquatic/marshland group usually have opposite or verticillate leaves that are narrow and sessile to sub-sessile, and small flowers (calyx usually 1–2 mm long, corolla usually less than 3 mm long) (Yao *et al.* 2016). Most of the species have a pair of opposite leaves at each node but among the species the number of leaves at each node ranges from three to more than ten, with four as the most common number. *Pogostemon cruciatus* (Benth.) Kuntze (1891: 530) (=*Dysophylla cruciata* Bentham (1830: 30)) and *P. stocksii* (Hook.f.) Press (1982: 74) (=*Dysophylla stocksii* Hooker (1885: 642)) have five leaves in a whorl; whereas in *P. deccanensis* (Panigrahi) Press (1982: 73) (=*Eusteralis deccanensis* Panigrahi (1976: 475)), *P. erectus* (Dalzell) Kuntze (1891: 530) (=*Dysophylla erecta* Dalzell (1850: 337)) and *P. stellatus* (Lour.) Kuntze (1891: 529) (=*Mentha stellata* Loureiro (1790: 361)) have eight or more leaves in a whorl (Bhatti & Ingrouille 1997).

In this paper, we propose a new species of *Pogostemon* from Konkan region of Maharashtra, India based on morphological and molecular data. The new species is closely allied to *Pogostemon deccanensis* and *P. erectus*. In addition to this, we have added molecular data of Indian endemic species of *Pogostemon* subg. *Dysophyllus* sect. *Verticillatus* and provided their phylogenetic placements.

Materials and method

Morphological study

During our survey in year 2015 to 2020, some specimens of *Pogostemon* were collected at the edges of natural ponds of lateritic plateaus around Karel, Kombe and Sakar of Konkan region of Maharashtra, India. Careful observations were made on the specimens in field as well as in laboratory under stereo-zoom microscope. The potential new species of *Pogostemon* were collected from Karel, Kombe and Sakar villages area of natural populations and compared with natural populations of *Pogostemon deccanensis* collected from Amboli, Bhuibawada and Dongar villages of Maharashtra state and *Pogostemon erectus* collected from Kurungwadi area, Igatpuri tehsil of Nashik District, Maharashtra and collected specimens of *Pogostemon* were submitted to Shivaji University (SUK) Kolhapur, Maharashtra, India. After examination of relevant literature (Cook 1996; Bhatti and Ingrouille 1997; Pradeep 1998) and critical analysis of vegetative parts of plants, all parts of flowers, fruits and mericarps of all specimens, it was determined that the new species is closely allied to *Pogostemon deccanensis* (Panigrahi) Press and *Pogostemon erectus* (Dalzell) Kuntze. In addition, specimens of *Pogostemon* were consulted at different herbaria of India (ASSAM, BSHC, BSI, BSJO, CAL, DRC, PBL, SUK). Details of studied materials are given in Table 1.

Molecular data

Total genomic DNA was extracted from young and fresh leaves of *Pogostemon deccanesis*, *P. erectus* and *P. jaitapurensis* using modified CTAB method (Paterson *et al.* 1993) with modifications described by Patil *et al.* (2015). The chloroplast DNA regions (*rbc*L, *mat*K, *trn*H-*psb*A and *trn*L-F) as well as nuclear ITS region have proved useful in previous phylogenetic studies within Lamiaceae (Bendiksby *et al.* 2011; Drew & Sytsma 2012; Roy *et al.* 2013; Yu *et al.* 2014; Yao *et al.* 2016). Hence, we have amplified and sequenced four chloroplast regions (*rbc*L, *mat*K, *trn*H-*psb*A and *trn*L-F) and nuclear ITS region from these three *Pogostemon* species. Primers used for amplification and sequencing of chloroplast DNA region and ITS regions were listed in Table 2. The polymerase chain reaction (PCR) for all selected markers were performed in 25 µL containing 12.5 µL AmpliTaq Gold® 360 Master mix (Applied Biosystem, USA), 8.5 µL Nuclease free water, 0.5 µL GC enhancer (Applied Biosystem, USA), 0.5 µL each forward and reverse primer

(15 μM μL^{-1}). PCR protocol was set to amplify all these selected marker as: 94°C for 4 min, 35 cycles (94°C for 30 s, 55°C for 1 min and 72°C for 1 min), and final extension was carried out at 72°C for 10 min. PCR reactions were carried out in Thermal cycler (BIO-RAD, USA). PCR products were checked on 1% agarose gel to confirm the amplification. Amplified PCR products were purified using GenElute™ PCR Clean-Up kit (SIGMA-ALDRICH, USA). Purified PCR products were sequenced bidirectionaly using BigDye® Terminator v 3.1 cycle sequencing kit (Applied Biosystems®) on 3500 Genetic Analyzer (Applied Biosystems, USA). Sequences of all selected regions were submitted to GenBank NCBI database.

DNA sequences from 37 taxa (69 accessions) matching all of the subgenera or sections that were circumscribed by Bhatii & Ingrouille (1997) and outgroups species were used for phylogenetic analyses. We used species of other genera in Lamiaceae such as *Anisomeles*, *Colebrookea*, *Craniotome*, *Eurysolen*, *Leucosceptrum*, *Microtoena*, *Colquhounia* and *Gomphostemma* as outgroups. Forty two sequences were generated in this study and added to the sequences generated by Yao *et al.* (2016) to carry out molecular analyses. Accession numbers for sequences generated in this study and retrieved from NCBI GeneBank database were provided in Table 1 and Supplementary material 1 (Table S1), respectively.

Sequence alignment and phylogenetic analyses

DNA Sequences were checked and edited by using Sequencher v 5.1 (Gene codes Corporation 2012) and multiple sequence alignment was carried out using MUSCLE (Edgar 2004) implemented in MEGA 7 (Kumar *et al.* 2016). Alignments of each gene then further refined using GBlocks v. 0.91b (Talavera & Castresana 2007). Three dataset (cpDNA, nrITS and combined cpDNA+ITS) were used for phylogenetic analyses. The sequence datasets were concatenated into single matrix using BioEdit v. 7.2.6 (Hall 1999). The incongruence among cpDNA data set (*rbc*L+*mat*K+ *trn*H-*psb*A+*trn*L-F) and nuclear ITS dataset was determined by using incongruence length test (ILD) (Farris *et al.* 1994) which was performed in PAUP 4.0a152 (Swofford 2002) with 100 homogeneity replicates, random taxon addition (holding 10 trees at each step), tree-bisection-swapping (TBR) searches, MULTREES option in effect with maxtree set to 100.

Phylogenetic analyses were carried out using two methods: Maximum Parsimony (MP) and Bayesian Inference (BI) for determining the phylogenetic position of new *Pogostemon* species and other two Indian endemic species. Maximum parsimony analyses were conducted in PAUP 4.0a152 (Swofford 2002) with 1000 bootstrap replicates. MP heuristic searches used 10 random taxon addition (holding 10 trees at each step) and Tree-bisection-reconnection (TBR) was used as branch-swapping algorithm, 'MulTrees' options was set in effect. Bayesian inference implementing Markov Chain Monte Carlo (MCMC) was conducted using MrBayes v.3.2.6 (Ronquist and Huelsenbeck 2003). Each dataset was treated as single partition, models of nucleotide substitution for cpDNA, nrITS and cpDNA+nrITS datasets were selected under the Akaike Information Criterion (AIC) using jModelTest 2 (Darriba *et al.* 2012) on XSEDE on the CIPRES science gateway (http://www.phylo.org/) (Miller *et al.* 2010). The models used were GTR+I+G for cpDNA, GTR+G for nrITS and GTR+I+G for combined (cpDNA+ITS) data (Table 3). Each MCMC analysis were run for 2,000,000 generations and sampling every 1000 generations. The first 25% trees were discarded as burn-in and the remainder were used to generate 50 % majority rule consensus bayesian tree having posterior probabilities (PPs) for each node. Average standard deviation of split frequencies at the end of run was below 0.01.

Results

Morphological analysis

It is revealed that the species of *Pogostemon* collected from Karel, Kombe, Sakar populations are clearly distinct from *P. deccanensis* and *P. erectus* (differences are listed in Table 3 and Fig. 1–2), and represents an undescribed species of *Pogostemon*. Hence it is described and illustrated here as a new species.

Phylogenetic analyses of combined cpDNA+nrITS *dataset*

The combined cpDNA+nrITS dataset included 37 taxa (69 accessions) contained 3150 characters, of which 2267 were conserved, 883 were variable and 725 were parsimony informative (Table 4). The results of incongruence

length difference test (ILD) between cpDNA and ITS dataset shows no significant incongruence in between them (P= 0.01), hence, we combined both. The phylogenetic analyses based on combined (cpDNA+ITS) data (Fig. 3) resolved the relationship within clade A and clade B with stronger support as compared to individual analyses based on cpDNA (Supplementary material 2, Fig. S2a) and ITS data (Supplementary material 2, Fig. S2b). Indian endemic species *P. deccanensis*, *P. erectus* and new species *P. jaitapurensis* were placed in *Pogostemon* subg. *Dysophyllus* sect. *Verticillatus*. All these species forms sister clade to African species *P. rogersii* Brown (1909: 379) with PP= 0.9 and BS= 77% (Fig. 1). Both *P. deccanensis* and *P. erectus* grouped together (PP= 1, BS= 100%) and *P. jaitapurensis* shows monophyly to them with PP= 0.99 and BS= 88%.

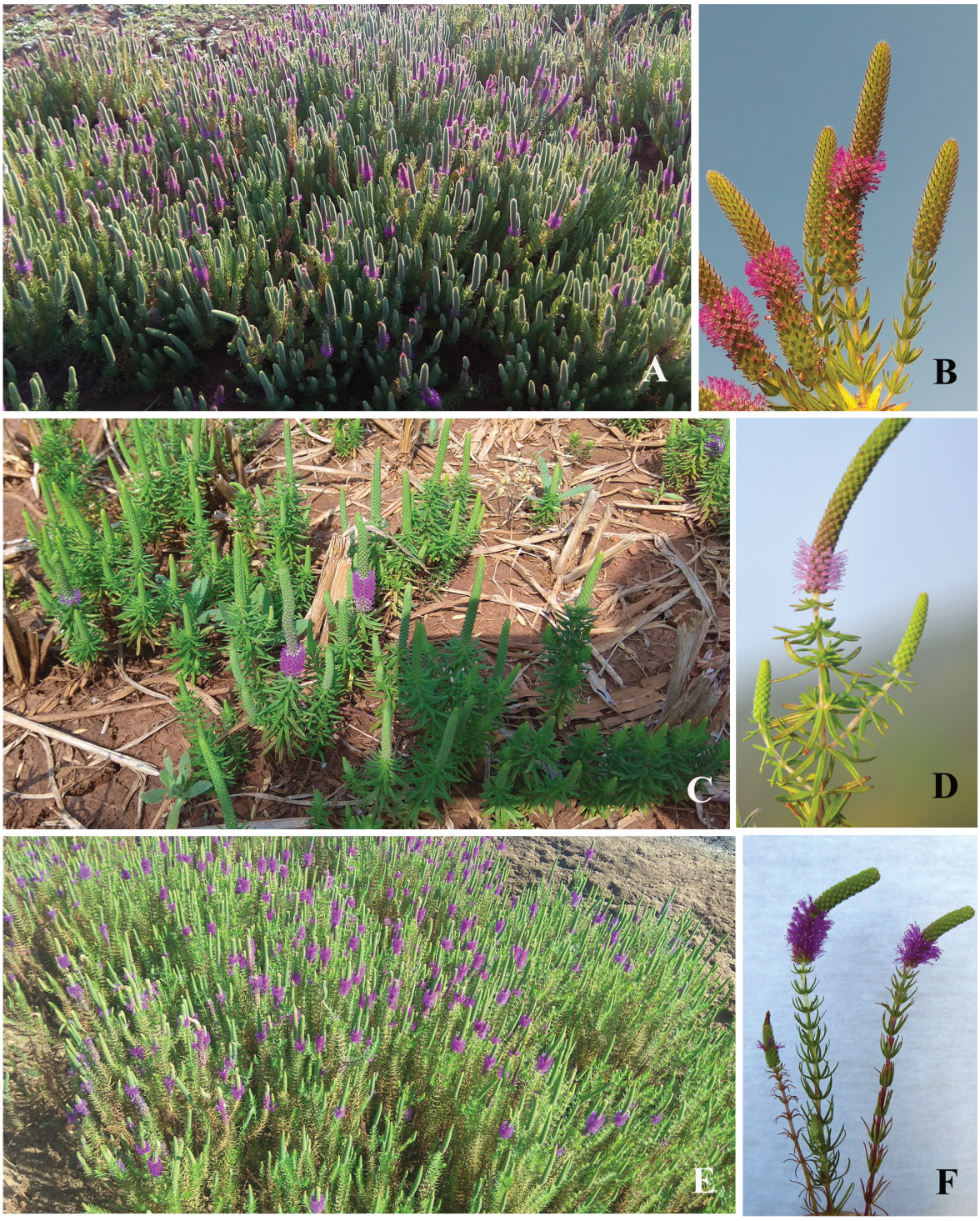

FIGURE 1. Habitat and habit comparison between *Pogostemon jaitapurensis* Chandore & S.R.Yadav, *sp. nov.*, *P. deccanensis* (Panigrahi) Press, and *P. erectus* (Dalzell) Kuntze. A & B *P. jaitapurensis* habitat; C & D *P. deccanensis* habitat; E & F *P. erectus* habitat. Photos by A.N. Chandore & S.R. Yadav.

TABLE 1. Details of studied plant species

Species	Area of collection	Location	Voucher ID's	*rbc*L	*mat*K	*trn*H-*psb*A	*trn*L-F	ITS
Pogostemon deccanensis (Panigrahi) Press	Kolape, Vaibhavawadi, Sindhudurg District, Maharashtra	N16°33.440 E73°46.244	ANC-1970	MH078517	MH420425	MH420431	MH078520	MH061384
			ANC-1972	MH420419	MH420426	MH420432	MH420440	MH411082
			ANC-1980	MH420420	MH420427	MH420433	MH420441	MH411083
Pogostemon erectus (Dalzell) Kuntze	Kurungwadi, Igatpuri, Nashik District, Maharashtra	N19°38.459 E73°40.574	ANC-2001	MH078518	-	MH420434	MH078521	MH061385
			ANC-2002	MH420421	-	MH420435	MH420442	MH411084
			ANC-2003	MH420422	-	MH420436	MH420443	MH411085
Pogostemon jaitapurensis Chandore & S.R. Yadav, *sp. nov.*	Karel, Rajapur, Ratnagiri District, Maharashtra,	N16°35.251 E73°24.713	ANC-1991	MH078519	MH420428	MH420437	MH078522	MH061386
			ANC-1994	MH420423	MH420429	MH420438	MH420444	MH411086
			ANC-1995	MH420424	MH420430	MH420439	MH420445	MH411087

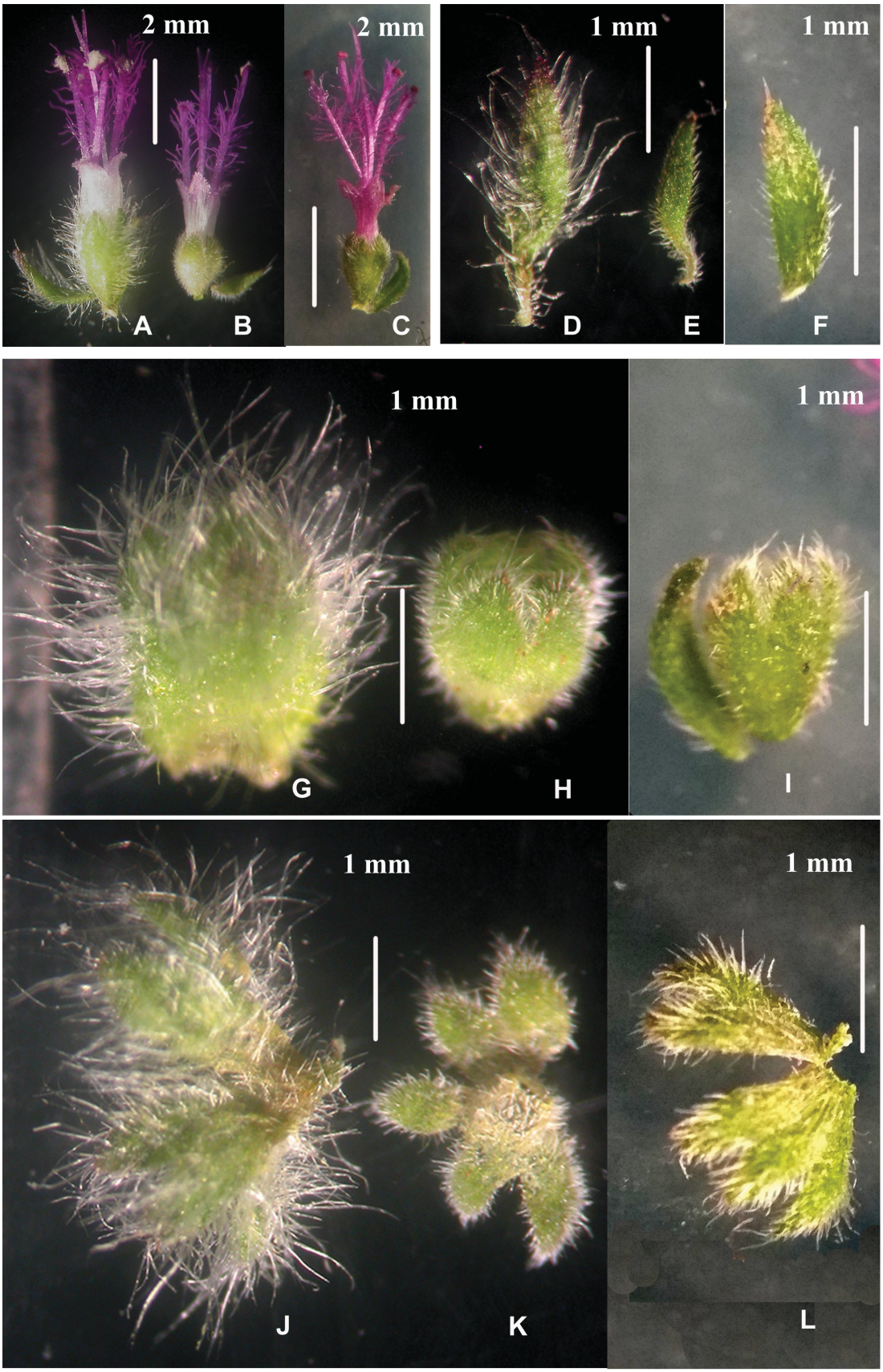

FIGURE 2. Comparison between *Pogostemon jaitapurensis* Chandore & S.R.Yadav, *sp. nov.*, *P. deccanensis* (Panigrahi) Press, and *P. erectus* (Dalzell) Kuntze. A. *P. jaitapurensis* flower; B. *P. deccanensis* flower; C. *P. erectus* flower; D. *P. jaitapurensis* bract; E. *P. deccanensis* bract; F. *P. erectus* bract; G. *P. jaitapurensis* calyx, H. *P. deccanensis* calyx; I. *P. erectus* calyx; J. *P. jaitapurensis* dissected calyx (outer surface); K. *P. deccanensis* dissected calyx (outer surface); L. *P. erectus* dissected calyx (outer surface). Photos by A.N. Chandore & S.R. Yadav.

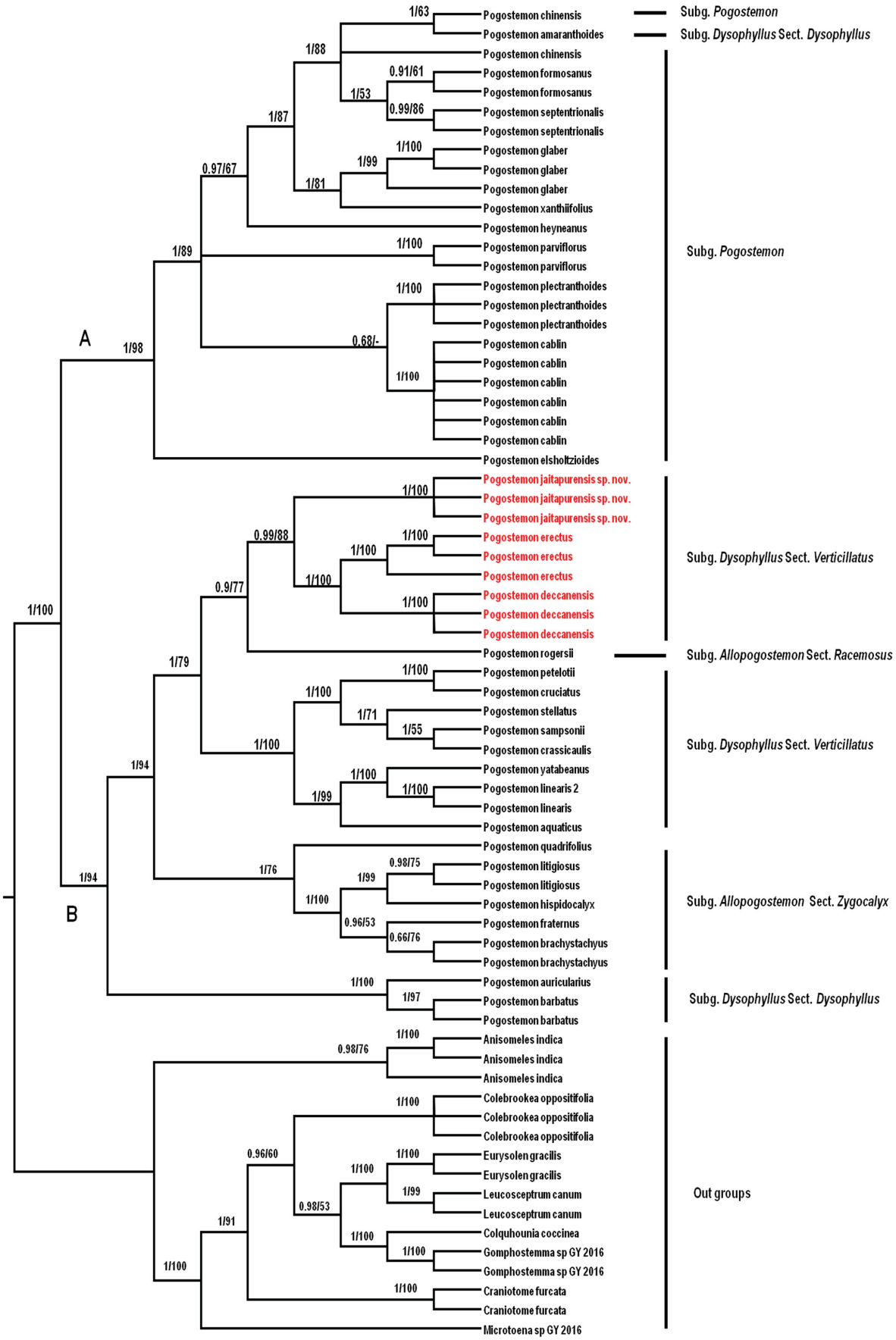

FIGURE 3. Bayesian 50% majority rule consensus phylogenetic tree based on combined cpDNA+ITS data. Bayesian posterior probability and maximum parsimony bootstrap (PP/MP BS) values provided next to corresponding branches. Species named in red colour are studied in this paper

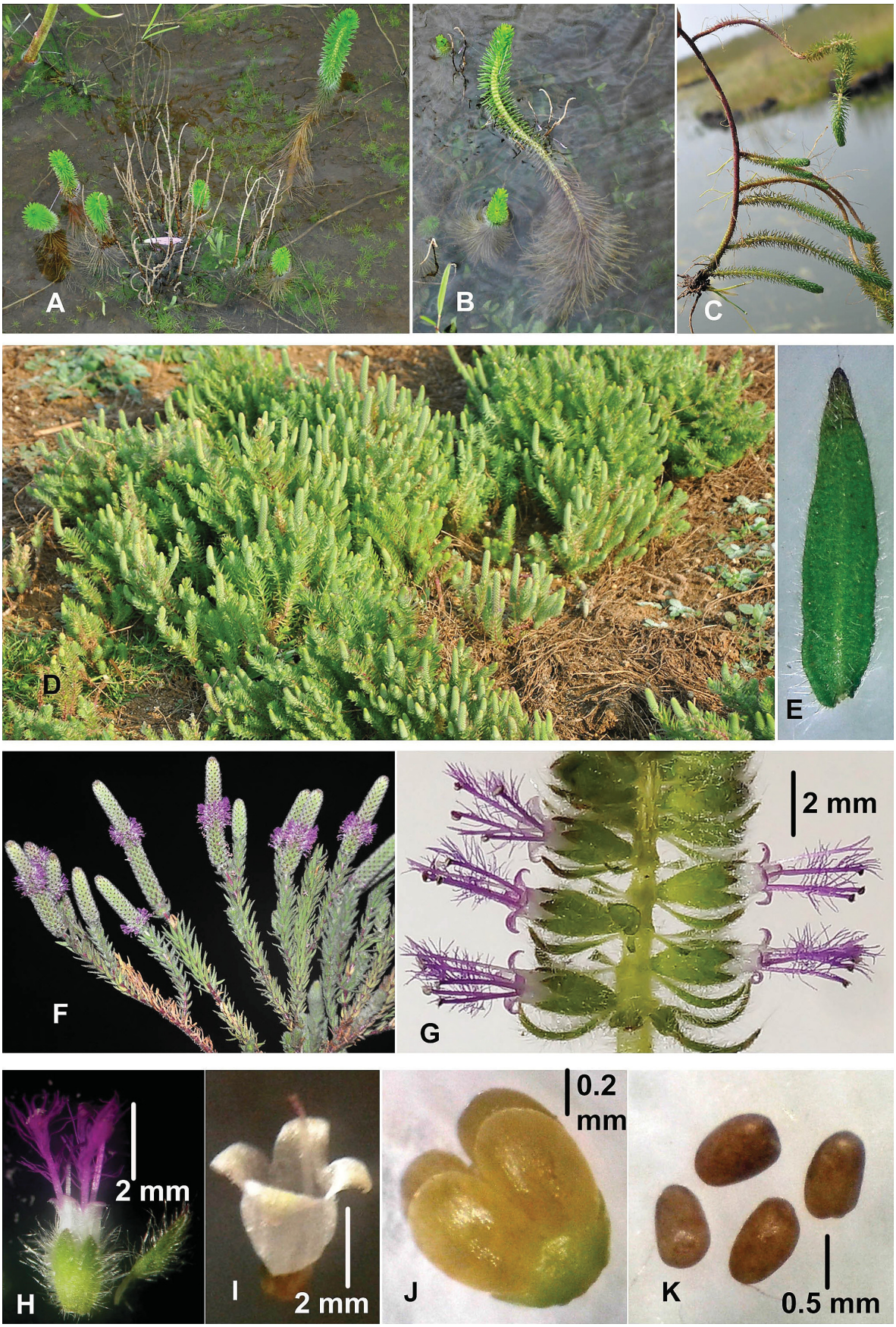

FIGURE 4. *Pogostemon jaitapurensis* Chandore & S.R.Yadav, *sp. nov.,* A–D Habitat; E. Leaf; F. Inflorescence; G. Close up to a inflorescence showing florets; H. Flower; I. Corolla without stamens; J. Ovary; K. Mericarps. Photos by A.N. Chandore & S.R. Yadav.

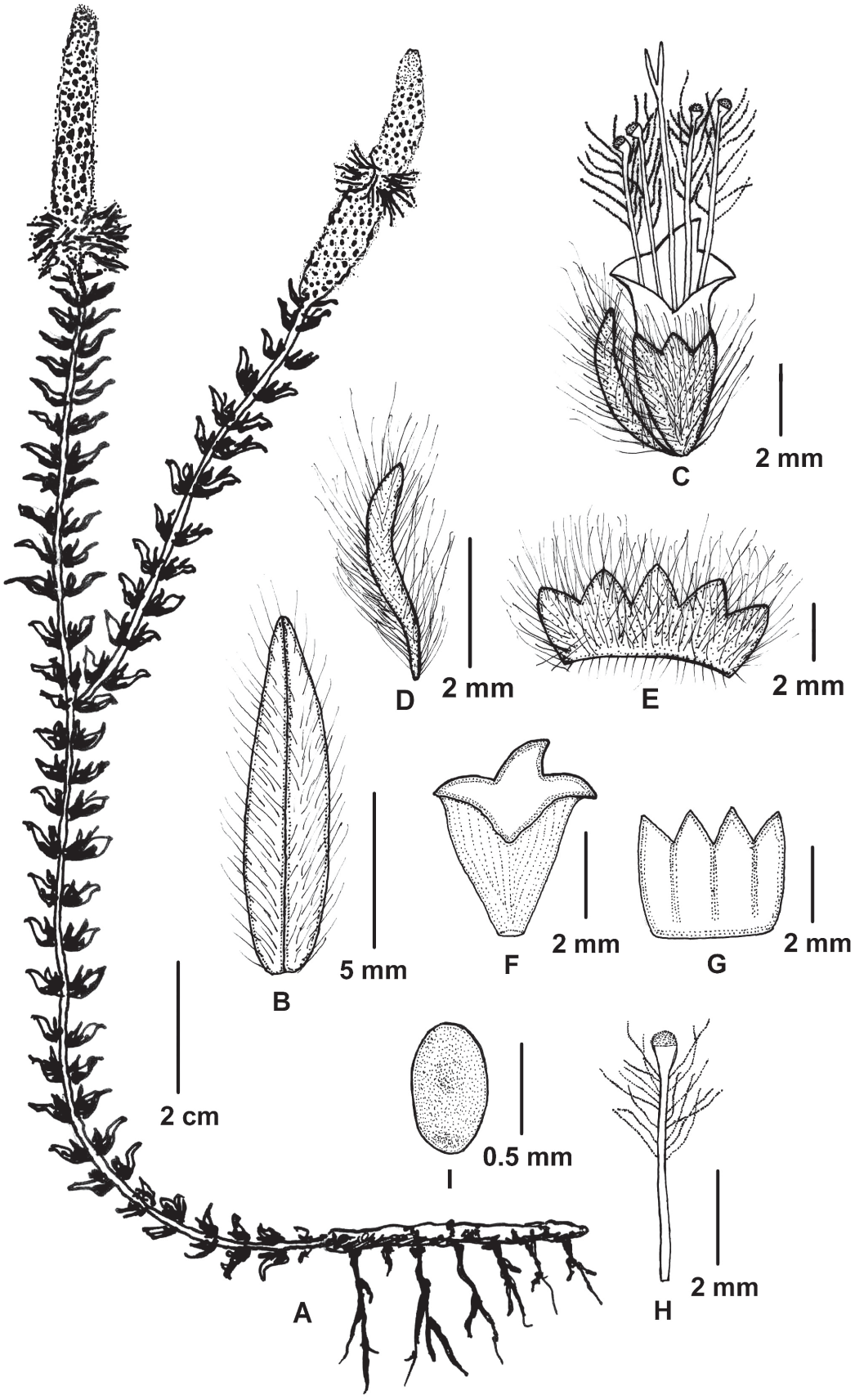

FIGURE 5. *Pogostemon jaitapurensis* Chandore & S.R.Yadav (*Chandore* 1991) A. Habit; B. Leaf; C. Flower; D. Bract; E. Calyx (outside); F. Corolla tube; G. Corolla lobes (outside); H. Filament with moniliform hairs; I. Mericarp. Illustration drawn by D.B. Borude.

TABLE 2. List of primers and their sequence used in this study

Marker	Primer	Sequence (5′ to 3′)	Reference
*rbc*L	rbcLa-F	ATGTCACCACAAACAGAGACTAAAGA	Levin *et al.* (2003) , modified from Soltis *et al.* (1992)
	rbcLa-R	GAAACGGTCTCTCCAACGCAT	Kress (2009) modified from Fofana *et al.* (1997)
matK	KIM-3F	CGTACAGTACTTTTGTGTTTACGAG	Kim, unpublished
	KIM-1R	ACCCAGTCCATCTGGAAATCTTGGTTC	Kim, unpublished
*trn*H-*psb*A	trnH2	CGCGCATGGTGGATTCACAATCC	Tate & Simpson (2003)
	psbAF	GTTATGCATGAACGTAATGCT	Sang *et al.* (1997)
*trn*L-F	*trn*L-c	CGAAATCGGTAGACGCTACG	Taberlet *et al.* (1991)
	*trn*F-f	ATTTGAACTGGTGACACGAG	Taberlet *et al.* (1991)
nrITS	ITSLeu1	GTCCACTGAACCTTATCATTTAG	Vargas *et al.* (1998)
	ITS4	TCCTCCGCTTATTGATATGC	White *et al.* (1990)

Phylogenetic analyses of cpDNA *dataset*

Totally, 37 taxa (69 accessions) including *Pogostemon* and outgroup species were used in cpDNA analyses. The combined four cpDNA region (*rbc*L+*mat*K+*trn*H-*psb*A+*trn*L-F) dataset contained 2542 characters, of which 2131 were conserved, 411 were variable and 308 were parsimony informative (Table 4). Bayesian Inference based on cpDNA dataset generated a topology similar to MP tree (Supplementary material 2, Fig. S2a–S2c). In *Pogostemon*, two main clades were resolved, Clade A (PP= 1, BS= 96%) and Clade B (PP= 1, BS= 99%). Phylogenetic relationship within Clade A was poorly resolved and some resolved nodes had weak support (Supplementary material 2, Fig. S2a). Clade A included species of *Pogostemon* subg. *Pogostemon* with *P. amaranthoides* Bentham (1848: 153) from subg. *Dysophyllus* sect. *Dysophyllus*. Clade B included accessions of *Pogostemon* subg. *Dysophyllus* sect. *Verticillatus*, subg. *Allopogstemon* sect. *Racemosus*, subg. *Dysophyllus* sect. *Zygocalyx* and subg. *Dysophyllus* sect. *Dysophyllus* (excluding *P. amaranthoides*). Based on cpDNA phylogeny newly added Indian endemic *Pogostemon* species namely *P. deccanensis*, *P. erectus* and new species *P. jaitapurensis* phylogenetically were placed in subgen. *Dysophyllus* sect. *Verticillatus.* All these species forms sister clade (PP= 1, BS= 52) to other species of that section (Supplementary material 2, Fig. S2a). *Pogostemon deccanensis* and *P. erectus* grouped with each other (PP= 1, BS= 93%) and *P. jaitapurenis* shows monophyly to both of the species with PP= 0.99 and BS= 99% (Supplementary material 2, Fig. S2a). *Pogostemon rogersii* from subgen. *Allopogostemon* sect. *Racemosus* showed monophyly to these three Indian endemic *Pogostemon* species with PP= 0.9 and BS= 97%.

Phylogenetic analyses of nrITS *dataset*

The aligned nrITS dataset included 36 taxa (67 accessions) contained 608 characters, of which 141 were conserved, 467 were variable and 417 were parsimony informative (Table 4). As in previous analysis by Yao *et al.* (2016) the African species *P. rogersii* from Subgen. *Allopogostemon* sect. *Racemosus* was excluded in the ITS phylogenetic analysis due to its substandard nrITS sequence quality, we also excluded it from our ITS analysis. Phylogenetic placements based on ITS data (Supplementary material 2, Fig. S2b) of all the subgenera and sections that were described by Bhatti and Ingrouille (1997) were similar with phylogenetic relationship shown by cpDNA dataset (Supplementary material 2, Fig. S2b). Phylogenetic relationship based on ITS data within Clade A was strongly supported (PP=0.91, BS= 96%) and that for Clade B was weakly supported (PP= 0.50, BS= 79%). Newly added Indian endemic species *P. deccanensis*, *P. erectus* and new species *P. jaitapurensis* were placed in subgen. *Dysophyllus* sect. *Verticillatus*, but there was difference in placement of both *P. deccanensis* and *P. erectus* species and in placement of *P. jaitapurenis* based on ITS data. *Pogostemon deccanensis* and *P. erectus* grouped together (PP= 1, BS= 100%) and showed monophyly to other species of sect. *Verticillatus* with PP= 0.50 and BS= 79% (Supplementary material 3, Fig. 3b). New species *P. jaitapurensis* grouped with other species of Sect. *Verticillatus* in different clade with forming sister clade to them with PP= 0.98 and BS= 95% (Supplementary material 2, Fig. S2b).

TABLE 3. Comparison of morphological features in *Pogostemon jaitapurensis sp. nov.*, *P. deccanensis* and *P. erectus*

Characters	*P. jaitapurensis sp. nov.*	*P. deccanensis*	*P. erectus*
Stem	creeping, up to 90 cm long; *ca.* 4 mm in diameter	creeping, up to 35 cm long; *ca.* 2.5 mm in diameter	erect, up to 30 cm long; *ca.* 2.0 mm in diameter
Stem internodes	densely hairy with long hairs	glabrous or short hairs	sparsely hairs
Leaves	linear-lanceolate, densely hairy throughout, apex acute	linear, glabrous or slightly hairy, apex obtuse or acute	narrowly linear, glabrous or minute hairy, apex acute
Floral bracts	linear to lanceolate, *ca.* 3.0 × 0.7 mm, hairs long, longest hairs up to 1.3 mm long	linear, *ca.* 1.2 × 0.3 mm, hairs very short, longest hairs up to 0.2 mm long	filiform to spathulate, *ca.* 1.5 × 0.35 mm, hairs short, longest hairs up to 0.25 mm long
Spike	densely hairy, 5–8 mm diameter in fruiting	minutely hairy, 3–4 mm in diameter in fruiting	minutely hairy, 3–4 mm in diameter in fruiting
Calyx	campanulate, *ca.* 2.2 × 0.4 mm, densely covered with long bulbous-based hairs; hairs up to 1.6 mm long	hemispheric or broadly campanulate, *ca.* 1.2 × 0.3 mm, sparsely covered with short bulbous-based hairs; hairs up to 0.2 mm long	campanulate, *ca.* 1.3 × 0.3 mm, covered with short bulbous-based hairs; hairs up to 0.4 mm long
Corolla	white, up to 3.2 mm long; lobes smooth outside	lilac to purple, up to 2.2 mm long; lobes hairy outside	purple, up to 2.3 mm long; lobes hairy outside
Filaments of moniliform hairs	up to 1.6 mm long	up to 0.5 mm long	up to 0.5 mm long
Mericarps	*ca.* 0.7 × 0.45 mm	*ca.* 0.5 × 0.3 mm	*ca.* 0.4 × 0.2 mm

TABLE 4. Characteristics of cpDNA, ITS and combined (cpDNA+ITS) data set

Characteristics	cpDNA	ITS	cpDNA+ITS
No. of taxa in analysis	37 taxa (69 accessions)	36 taxa (67 accessions)	37 taxa (69 accessions)
Aligned length (bp)	2542	608	3150
Conserved sites (cs)	2131	141	2267
Variable sites (vs)	411	467	883
Parsimony informative sites (pis)	308	417	725
Best fit nucleotide model	GTR+I+G	GTR+G	GTR+I+G

Discussion

Morphological study

In *Pogostemon* subg. *Dysophyllus* sect. *Verticillatus, P. deccanensis*, *P. erectus* and *P. stocksii* are endemic to India. Morphologically *P. jaitapurensis* sp. nov. is very similar to *P. deccanensis* and *P. erectus*. Corolla lobes of *P. jaitapurensis* are smooth, while in *P. deccanensis* and *P. erectus* the lobes are hairy outside (Fig. 2). In *P. jaitapurensis* the calyx and bracts are densely hairy and the hairs are much long, these diagnostic characters are not found in *Pogostemon* sect. *Verticillatus*. *Pogostemon jaitapurensis* is easily distinguishable from *P. deccanensis* and *P. erectus*, mainly in the corolla architecture (Fig. 2). Corolla is white in colour and up to 3.2 mm long in *P. jaitapurensis*, while corolla is lilac to purple and up to 2.2 mm long in *P. deccanensis* and corolla purple in colour, up to 2.3 mm long in *P. erectus*. The filaments of moniliform hairs are longer in *P. jaitapurensis* (1.6 mm) as compared to *P. deccanensis* and *P. erectus* (0.5 mm long). The dimensions of mericarps also larger in *P. jaitpaurensis* as compared to *P. deccanensis* and *P. erectus* (Table 3).

Phylogenetic analyses and phylogenetic positions of Indian endemic Pogostemon species

The present study added the three Indian endemic *Pogostemon* species of subg. *Dysophyllus* sect. *Verticillatus* and evaluated their phylogenetic positions. Our phylogenetic analyses largely agreed with the study of Yao *et al.* (2016) that *Pogostemon* is sister to *Anisomeles*. The relationship of all subgenera or sections circumscribed by Bhatti & Ingrouille (1997) was also found similar with that shown by Yao *et al.* (2016) even after the addition of some Indian species from sect. *Verticillatus.* Phylogenetic analyses based on cpDNA, nrITS and combined cpDNA+ITS dataset resolved *Pogostemon* into two clades. Clade A contained species of Subgen. *Pogostemon* + *P. amaranthoides* (subg. *Dysophyllus* sect. *Dysophyllus*). Clade B included species of subg. *Dysophyllus* sect. *Verticillatus*, subg. *Allopogostemon* sect. *Racemosus*, subg. *Allopogostemon* sect. *Zygocalyx* and subg. *Dysophyllus* sect. *Dysophyllus* (excluding *P. amaranthoides*) (Fig. 3 Supplementary material 2, Fig. S2a, S2b).

All the Bayesian and parsimony phylogenetic analyses based on cpDNA, nrITS and combined (cpDNA+ITS) dataset revealed that newly added Indian endemic species and new *Pogostemon* species belong to Clade B and grouped with the species of *Pogostemon* subg. *Dysophyllus* sect. *Verticillatus* with strong support (Fig. 3 and Supplymentary material 2, Fig. S2a–S2e). *Pogostemon deccanensis* and *P. erectus* found to be grouped together in phylogenetic analyses of all the three datasets. The new species, *P. jaitapurensis*, was recovered as monophyletic in both analyses of cpDNA and combined (cpDNA+ITS) datasets (Fig. 3, Supplementary material 2, Fig. S2a). But ITS phylogeny shows some difference in the placement of *P. deccanensis*, *P. erectus* species and *P. jaitapurenis* (Fig. S2b). It means cpDNA and combined (cpDNA+ITS) datasets distinguished *P. jaitapurenis* from *P. deccanensis* and *P. erectus*. *Pogostemon deccanensis* and *P. erectus* were already placed within sect. *Verticillatus*, and now *P. jaitapurensis* is also added to this section in accordance to its phylogenetic position (Fig. 3, Supplementary material 3, Fig. S2a, S2b).

Taxonomic Treatment

Pogostemon jaitapurensis Chandore & S.R.Yadav, *sp.nov.* (Figs. 1, 2, 4 and 5)

Type:—INDIA, Maharashtra, Ratnagiri District, Rajapur, near to Jaitapur, Karel, 16°35'251''N, 73°24'713''E, 71 m, 3 December 2015, *A.N.Chandore 1991* (holotype CAL!, isotypes BSI!, K!, SUK!).

Diagnosis:—*Pogostemon jaitapurensis* is similar to *Pogostemon deccanensis*, but differs in its densely long hairs on stem internodes (*vs.* glabrous or short hairs on stem internodes), leaves linear to lanceolate, throughout densely hairy (*vs.* linear, glabrous or slightly hairy), bracts linear to lanceolate, *ca.* 3 × 0.7 mm, long hairs, longest hairs up to 1.3 mm (*vs.* bracts linear, *ca.* 1.2 × 0.3 mm, very short hairs, longest hairs up to 0.2 mm), calyx campanulate with long bulbous-based hairs, hairs up to 1.6 mm long (*vs.* hemispheric or broadly campanulate with short bulbous-based hairs, hairs up to 0.2 mm long), corolla glabrous outside, white, up to 3.2 mm long (*vs.* corolla hairy outside, lilac to purple, up to 2.2 mm long), filaments of moniliform hairs up to 1.6 mm long (*vs.* moniliform hairs up to 0.5 mm long). Other differences between *Pogostemon jaitapurensis* and *P. deccanensis* and *P. erectus* are listed in Table 3.

Perennial herbs with woody rootstock and fibrous roots, 7–25 cm long. Stem creeping, 45–90 cm long, 3.8–4.3 mm in diameter, decumbent, much branched, flexible with short internodes, strigose hairy, rooting at the nodes; erect part 8–25 cm long, 1–4 mm in diameter, with dense white strigose hairs. Leaves sessile, in whorls of 6–12, linear-lanceolate, 5–10 × 1.3–2.3 mm, densely hairy throughout, (submerged leaves 12–20 at each whorl, filiform, 3–18 × 0.2–0.6 mm), margin entire, midrib prominent, tip acute. Spikes 3–7 cm long; 5–8 mm in diameter in fruiting; bracts linear to lanceolate, always longer than calyx, 2.5–3.3 mm long, 0.65–0.78 mm width, densely strigose hairy, longest hair up to 1.3 mm, persistent. Flowers dense arranged in spiciform inflorescences; calyx campanulate, 5-lobed, lobes connate at the base, deltate at apex, subequal, linear to lanceolate, 2.1–2.3 × 0.38–0.42 mm, densely strigose hairy, hairs 1.3–1.6 mm long, translucent or whitish, tube 1.4–1.6 mm long; corolla 4-lobed, 3.0–3.2 mm long, white, glabrous; lobes subequal, smooth outside, ovate, tube 2.3–2.5 mm long; stamens 4, equal, filaments 4.5–5 mm long, lavender in upper half, translucent or whitish in lower half with long moniliform hairs from the middle to connective of anthers, anthers 1-locular; ovary 5.8–6.2 mm long, 4-lobed, style bifid. Mericarps oblong to ovoid, 0.68–0.75 × 0.43–0.47 mm, pale yellowish-brown, smooth and shining.

Distribution:—Endemic to India, occurring in Maharashtra; Konkan region, Ratnagiri district, Rajapur Tehsil, Karel, Kombe and Sakar.

Habitat:—*Pogostemon jaitapurensis* grows on lateritic plateaus of Konkan in and along the edges of natural ponds at an elevation of about 71 m in association with *Aeschynomene aspera* Linnaeus (1753: 713), *Aponogeton nateshii* Yadav (2015: 9), *Blyxa* sp., *Corynandra elegans* Chandore *et al.* (2016: 89), *Cryptocoryne* sp., *Cyperus pulchellus* Brown (1810: 213), *Eleocharis konkanensis* Chandore *et al.* (2016: 154) and *Eriocaulon rayatianum* Chandore *et al.* (2019: 73).

Phenology:—Flowering and Fruiting from November to February.

Etymology:—The specific epithet "*jaitapurensis*" is given after the place Jaitapur in Ratnagiri district of Maharashtra.

Conservation Status:—The occurrence of *Pogostemon jaitapurensis* is estimated to be less than 2 km^2 of Karel, Kombe & Sakar, where a new Nuclear Power Project is being developed which could be major threat for the survival of the species. It is assessed here as Critically Endangered (CR, B1b (iii, v)) (IUCN 2019).

Additional specimens examined (Paratypes):—INDIA, Maharashtra, Ratnagiri District, Rajapur Tahsil, Karel, 14 December 2015, *A.N.Chandore 1997* (SUK!); Kombe, 15 December 2015, *A.N.Chandore 1998* (SUK!); Sakar, 15 December 2015, *A.N.Chandore 1999* (SUK!).

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Supplementary material 1

TABLE S1. List of taxa used for molecular analyses and their accession numbers

Taxa	GenBank accession numbers				
	rbcL	*mat*K	*trn*H-*psb*A	*trn*L-F	ITS
Pogostemon* Subg. *Pogostemon					
Pogostemon cablin	KR608503	KR608438	KR608562	KR608690	KR608757
Pogostemon cablin	KR608504	KR608439	KR608563	KR608691	KR608752
Pogostemon cablin	KR608505	KR608440	KR608564	KR608692	KR608753
Pogostemon cablin	KR608506	KR608441	KR608565	KR608693	KR608754
Pogostemon cablin	KR608507	KR608442	KR608566	KR608694	KR608755
Pogostemon cablin	KR608508	KR608443	KR608567	KR608695	KR608756
Pogostemon chinensis	KR608491	KR608426	KR608550	KR608678	KR608743
Pogostemon chinensis	KR608512	KR608449	KR608573	KR608699	KR608742
Pogostemon elsholtzioides	-	KR608445	KR608569	KR608720	-
Pogostemon formosanus	KR608499	KR608434	KR608558	KR608686	KR608744
Pogostemon formosanus	KR608500	KR608435	KR608559	KR608687	KR608779
Pogostemon glaber	KR608496	KR608431	KR608555	KR608683	KR608740
Pogostemon glaber	KR608494	KR608429	KR608553	KR608681	KR608739
Pogostemon glaber	KR608495	KR608430	KR608554	KR608682	KR608741
Pogostemon heyneanus	KR608492	KR608427	KR608551	KR608679	KR608751
Pogostemon parviflorus	KR608501	KR608436	KR608560	KR608688	KR608749
Pogostemon parviflorus	KR608502	KR608437	KR608561	KR608689	KR608750
Pogostemon plectranthoides	KR608509	KR608446	KR608570	KR608696	KR608760
Pogostemon plectranthoides	KR608510	KR608447	KR608571	KR608697	KR608758
Pogostemon plectranthoides	KR608511	KR608448	KR608572	KR608698	KR608759
Pogostemon septentrionalis	KR608497	KR608432	KR608556	KR608684	KR608747
Pogostemon septentrionalis	KR608498	KR608433	KR608557	KR608685	KR608748
Pogostemon xanthiifolius	KR608493	KR608428	KR608552	KR608680	KR608746
Pogostemon* Subg. *Allopogostemon* Sect. *Zygocalyx					
Pogostemon brachystachyus	KR608517	KR608455	KR608579	KR608704	KR608775
Pogostemon brachystachyus	KR608516	KR608455	KR608578	KR608703	KR608774
Pogostemon fraternus	-	KR608461	KR608585	KR608710	KR608781
Pogostemon hispidocalyx	-	KR608457	KR608581	KR608706	KR608780
Pogostemon litigiosus	KR608519	KR608458	KR608582	KR608707	KR608776
Pogostemon litigiosus	KR608520	KR608459	KR608583	KR608708	KR608777
Pogostemon* Subg. *Allopogostemon* Sect. *Racemosus					
Pogostemon rogersii	-	KR608460	KR608584	KR608709	KR608782
Pogostemon* Subg. *Dysophyllus* Sect. *Dysophyllus					
Pogostemon amaranthoides	KR608490	KR608425	KR608549	KR608677	KR608745
Pogostemon auricularius	KR608513	KR608451	KR608575	KR608700	KR608761
Pogostemon barbatus	KR608514	KR608452	KR608576	KR608701	KR608762
Pogostemon barbatus	KR608515	KR608453	KR608577	KR608702	KR608763
Pogostemon quadrifolius	KR608518	KR608456	KR608580	KR608705	KR608773
Pogostemon* Subg. *Dysophyllus* Sect. *Verticillatus					

Pogostemon aquaticus	KR608527	KR608468	KR608592	KR608717	KR608767
Pogostemon crassicaulis	KR608528	KR608469	KR608593	KR608718	KR608770
Pogostemon cruciatus	KR608525	KR608466	KR608590	KR608715	KR608771
Pogostemon linearis	KR608521	KR608462	KR608586	KR608711	KR608764
Pogostemon linearis	KR608522	KR608463	KR608587	KR608712	KR608765
Pogostemon petelotii	KR608529	KR608470	KR608594	KR608719	KR608772
Pogostemon sampsonii	KR608524	KR608465	KR608589	KR608714	KR608769
Pogostemon stellatus	KR608523	KR608464	KR608588	KR608713	KR608768
Pogostemon yatabeanus	KR608526	KR608467	KR608591	KR608716	KR608766
Outgroups					
Anisomeles indica	KR608471	KR608406	KR608530	KR608658	KR608726
Anisomeles indica	KR608473	KR608408	KR608532	KR608660	KR608727
Anisomeles indica	KR608474	KR608409	KR608533	KR608661	KR608728
Colebrookea oppositifolia	KR608479	KR608414	KR608538	KR608666	KR608732
Colebrookea oppositifolia	KR608480	KR608415	KR608539	KR608667	KR608733
Colebrookea oppositifolia	KR608481	KR608416	KR608540	KR608668	KR608734
Craniotome furcata	KR608477	KR608412	KR608536	KR608664	KR608730
Craniotome furcata	KR608478	KR608413	KR608537	KR608665	KR608731
Eurysolen gracilis	KR608482	KR608417	KR608541	KR608669	KR608735
Eurysolen gracilis	KR608483	KR608418	KR608542	KR608670	KR608736
Leucosceptrum canum	KR608484	KR608419	KR608543	KR608671	KR608738
Leucosceptrum canum	KR608485	KR608420	KR608544	KR608672	KR608737
Microtoena sp. GY-2016	KR608475	KR608410	KR608534	KR608662	KR608729
Colquhounia coccinea	KR608486	KR608421	KR608535	KR608673	KR608722
Gomphostemma sp. GY-2016	KR608487	KR608422	KR608545	KR608674	KR608723
Gomphostemma sp. GY-2016	KR608488	KR608423	KR608546	KR608675	KR608724

Supplementary material 2. Bayesian and Maximum parsimony phylogenetic trees based on cpDNA, ITS and combined (cpDNA+ITS) dataset

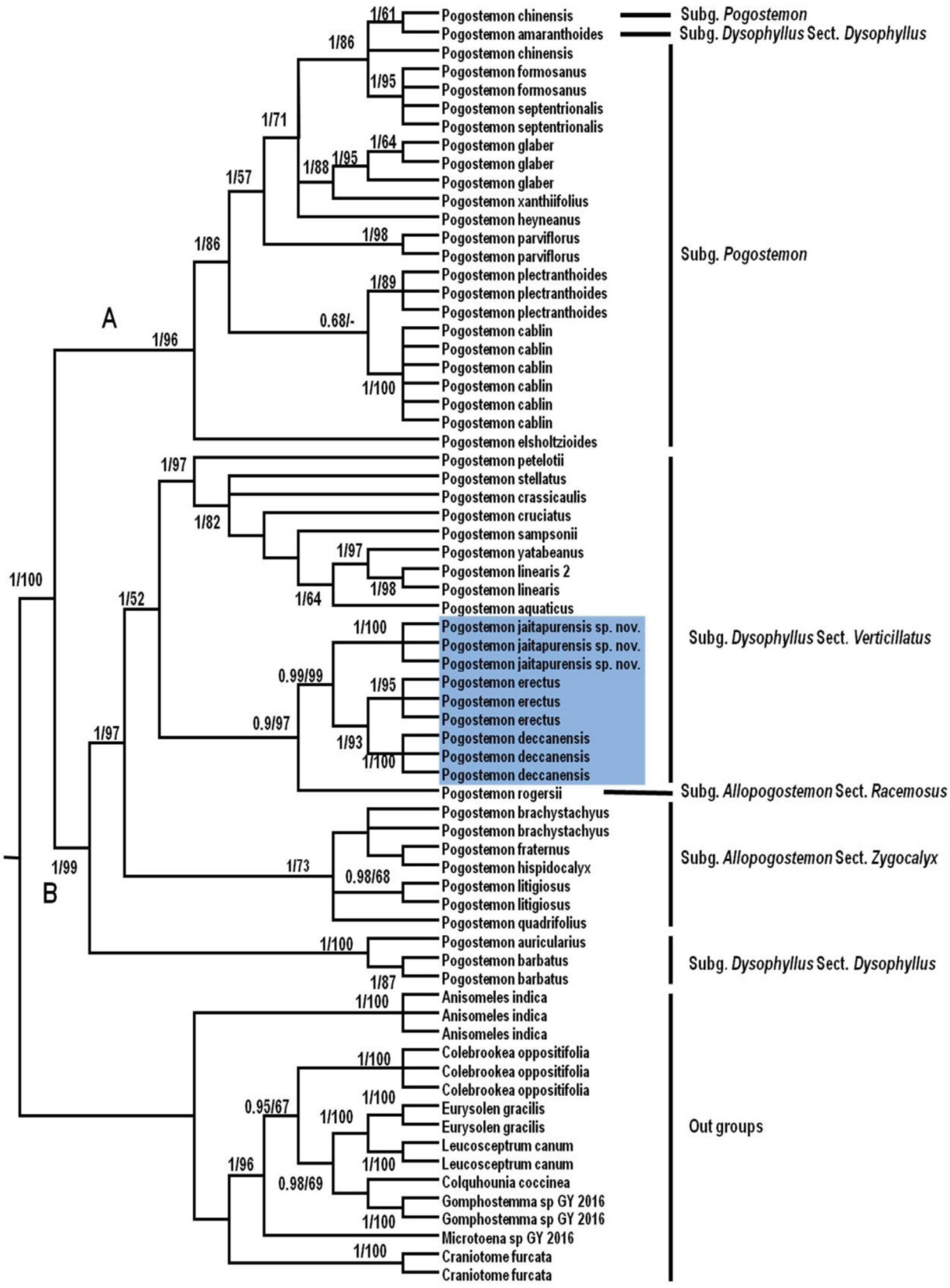

FIGURE S2a. Bayesian 50% majority rule consensus phylogenetic tree based on combined cpDNA dataset (*rbc*L+*mat*K+*trn*H-*psb*A+*trn*L-F). Bayesian posterior probability and maximum parsimony bootstrap (PP/MP BS) values provided next to corresponding branches.

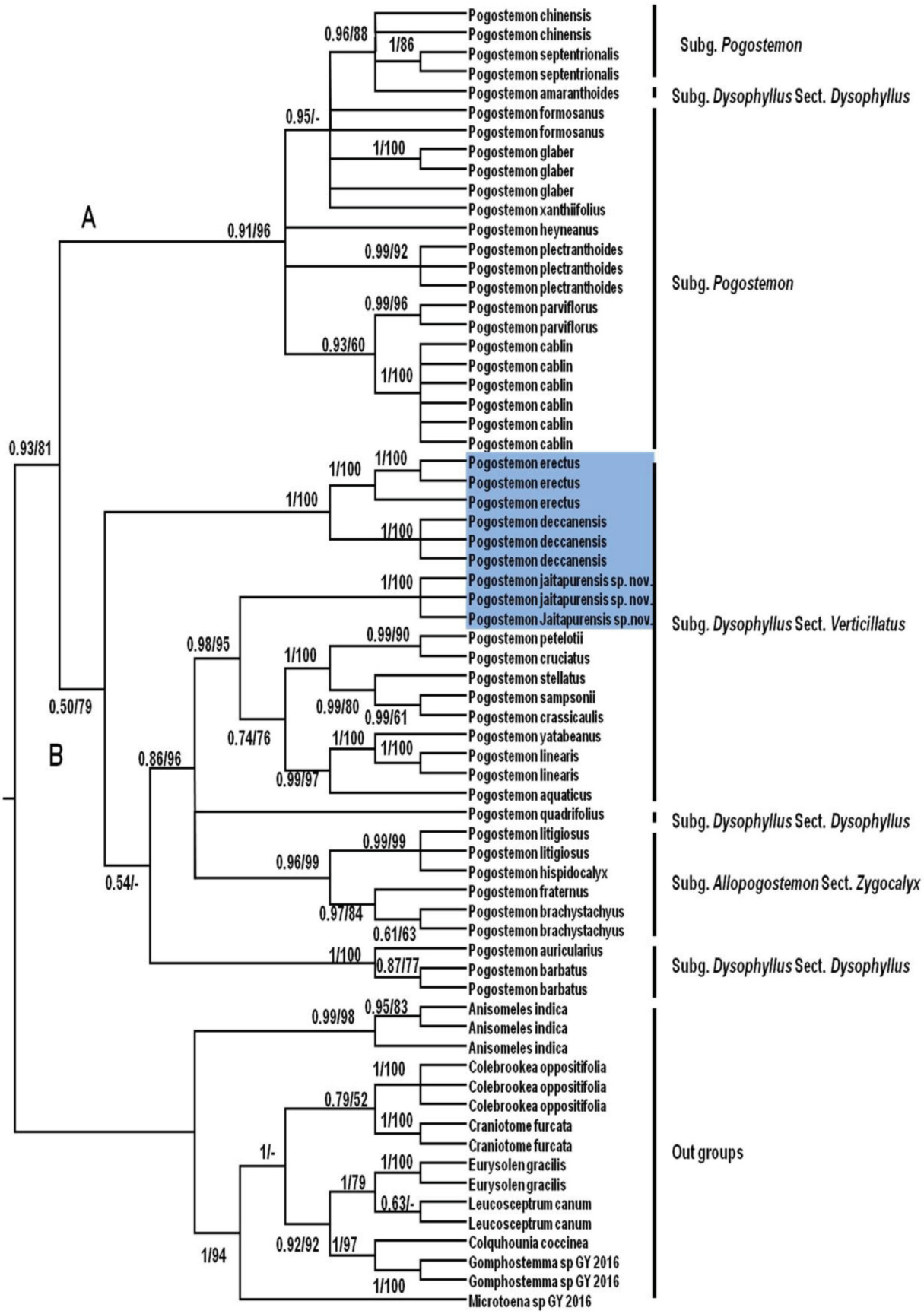

FIGURE S2b. Bayesian 50% majority rule consensus phylogenetic tree based on ITS dataset Bayesian posterior probability and maximum parsimony bootstrap (PP/MP BS) values provided next to corresponding branches.

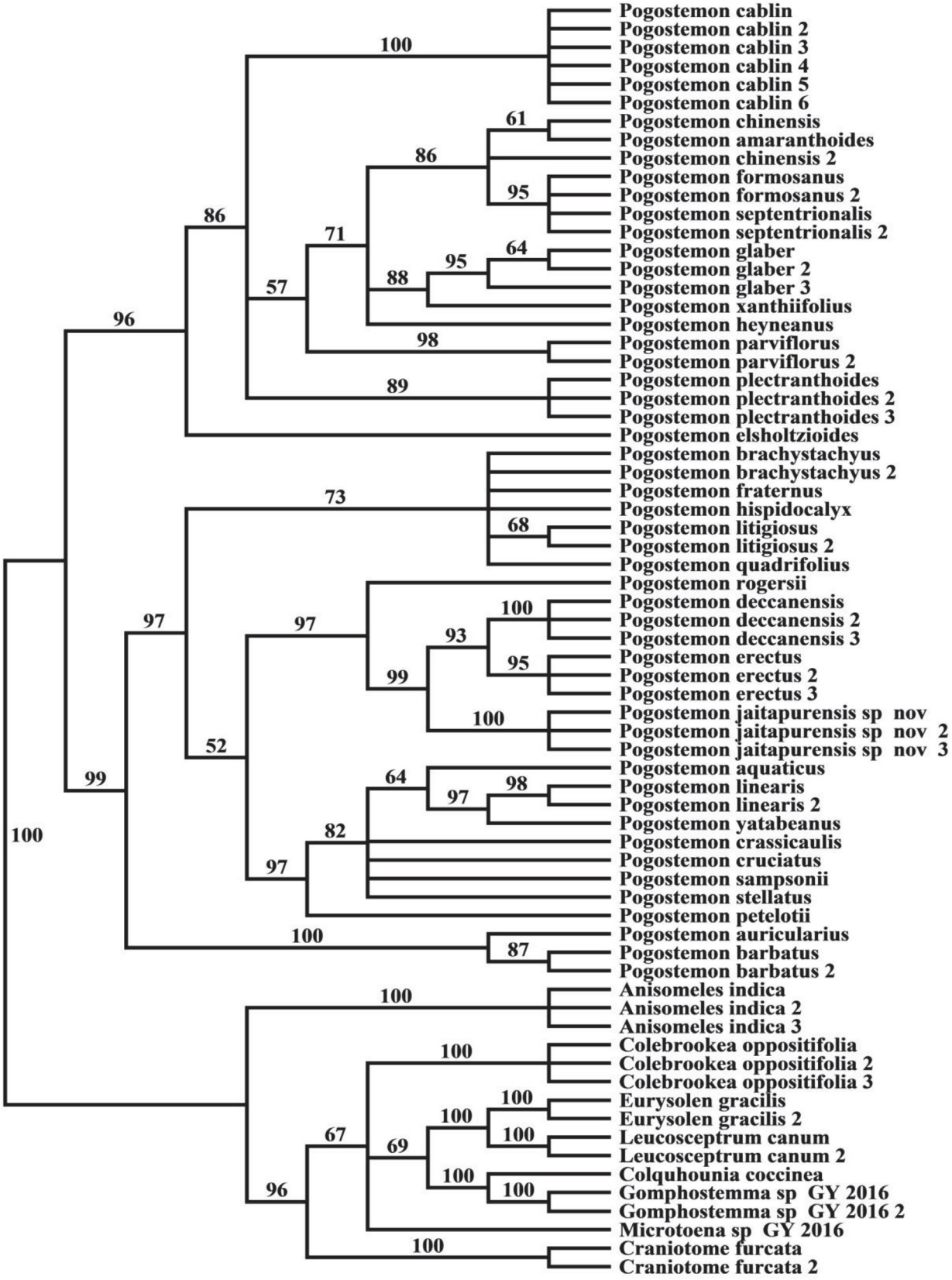

FIGURE S2c. 50% majority rule consensuses parsimony tree based on combined cpDNA dataset. The most parsimonious tree with length = 536 is shown. The consistency index= 0.832 , the retention index= 0.957. The percentage of replicate tree in which associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

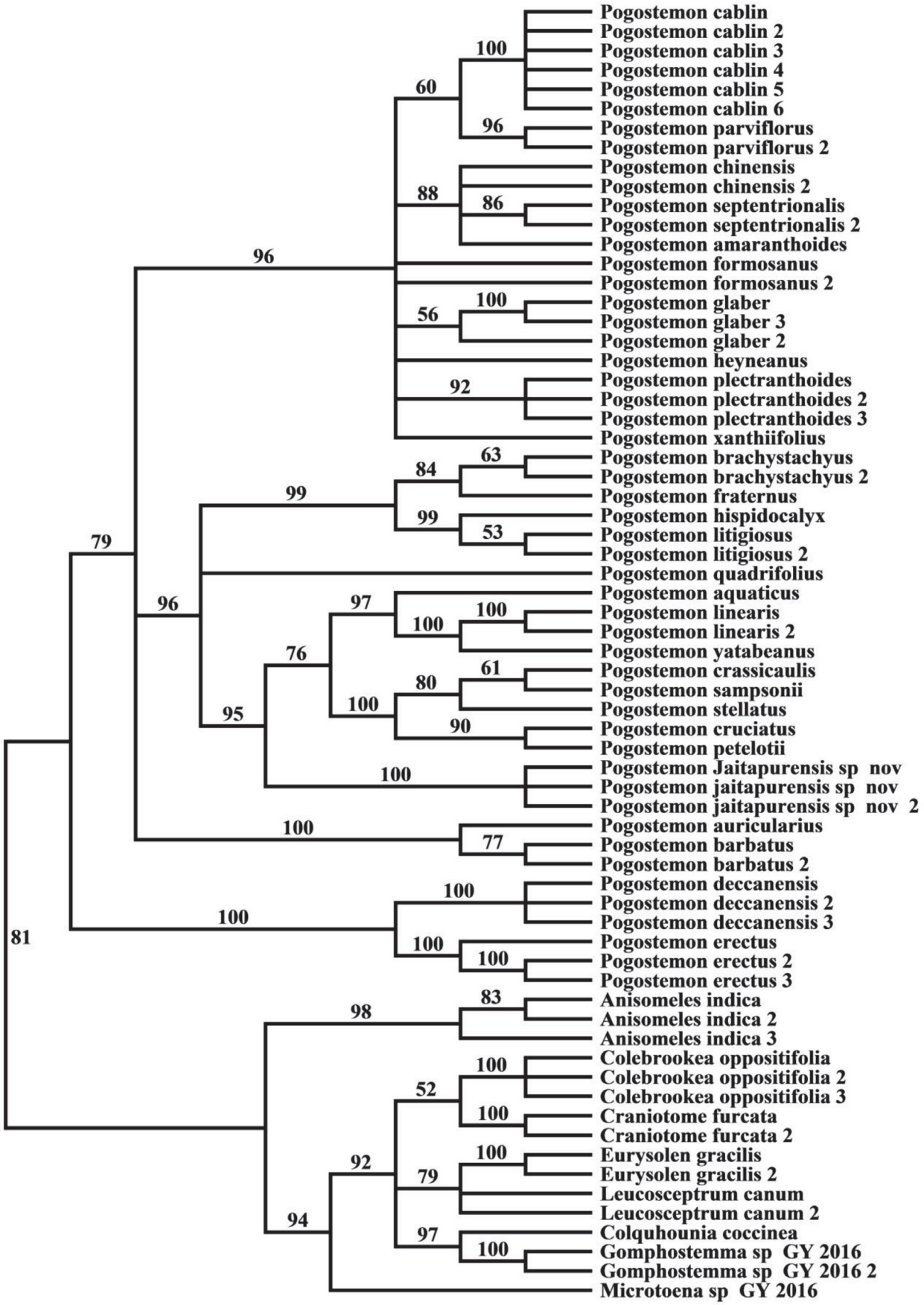

FIGURE S2d. 50% majority rule consensuses parsimony tree based on ITS dataset. The most parsimonious tree with length = 1272 is shown. The consistency index= 0.573, the retention index= 0.837. The percentage of replicate tree in which associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

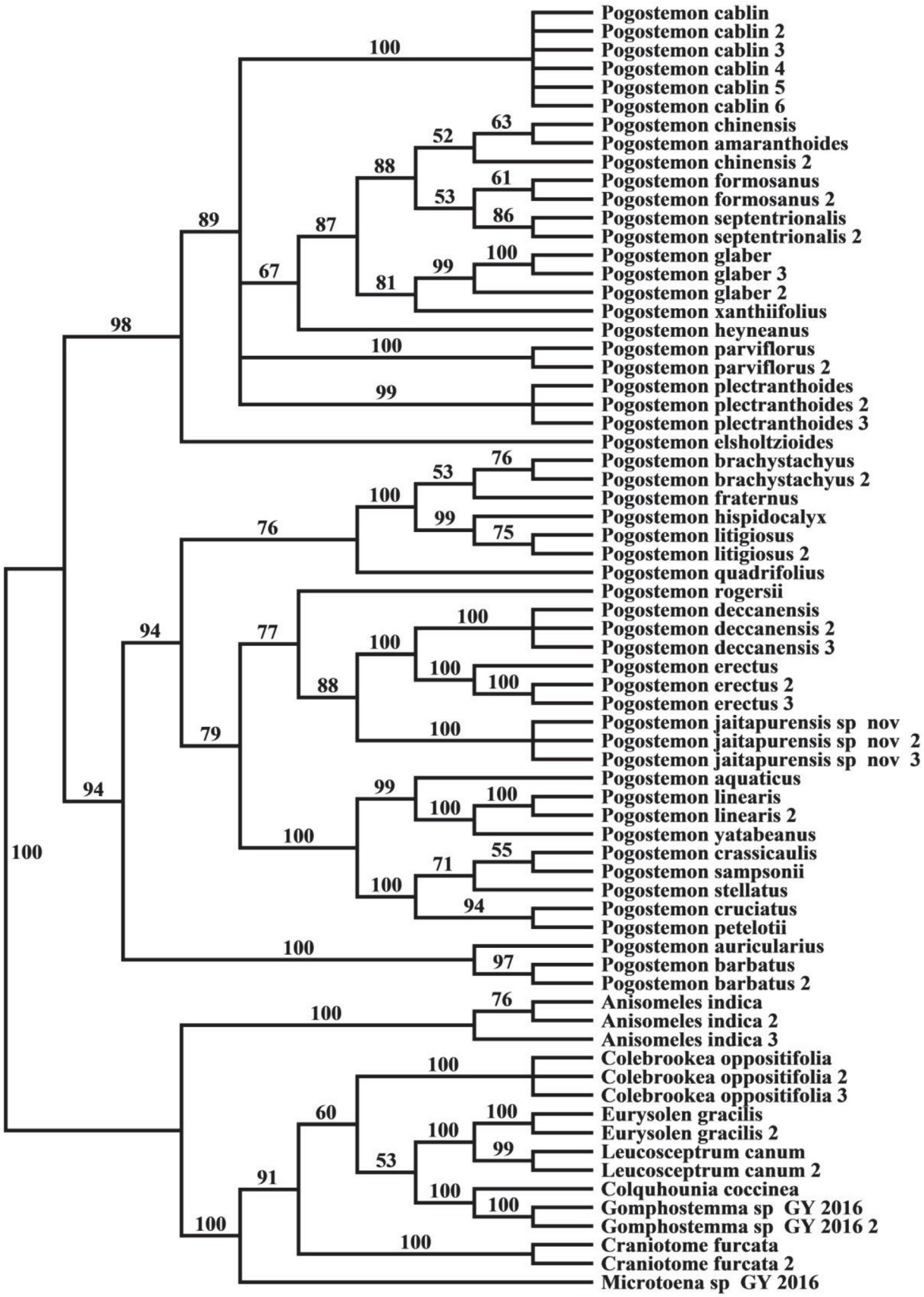

FIGURE S2e. 50% majority rule consensuses parsimony tree based on combined cpDNA+ITS dataset. The most parsimonious tree with length = 1831 is shown. The consistency index= 0.648, the retention index= 0.881. The percentage of replicate tree in which associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.